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Effects of Drought Stress on Chlorophyll Fluorescence Parameters, Chlorophyll Content and Grain Yield of Wheat Cultivars

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Abstract: Chlorophyll fluorescence quick variation can be used as a valuable index for evaluation of plants tolerance to environmental stresses. In order to evaluate chlorophyll quick fluorescence fluctuations of different wheat cultivars under water-limited condition, a factorial experiment with a randomized complete block design was performed in Karaj, Iran. Treatments were seven different irrigation regimes and three bread wheat cultivars with four replications. The measurements of Chlorophyll fluorescence parameters were done on flag leaves about three weeks after flowering. Photo-system II photochemical capacity was calculated from the ratio of variable fluorescence to maximum chlorophyll fluorescence (FV/FM). In addition, $T_{1/2}$ and FV were evaluated and relative water content and flag leaf chlorophyll were also measured. Results showed that different irrigation levels affected the FV, FV/FM and $T_{1/2}$ significantly ($p < 0.05$), but have not any significant effect on F0 and FM. There was a significant difference between different varieties and irrigation levels in respect to chlorophyll content, RWC and grain. The means of FV/FM, FV, $T_{1/2}$ and FM were declined as soil water content was decreased, but F0 was almost remained constant for all the treatments. High yielding varieties had higher $T_{1/2}$, FM, FV/FM, FV, chlorophyll content and RWC values. The FV and FV/FM had highest and F0 had lowest correlation coefficients with grain yield. The existing synchronized pattern of variation in fluorescence parameters of all varieties indicates that high yielding varieties can avoid the negative effects of drought stress during grain filling period. The high correlation between fluorescence parameters and RWC confirm these findings.

Key words: Wheat, limited irrigation, drought stress, chlorophyll fluorescence, relative water content

INTRODUCTION

Drought stress is one of the most important environmental factors that limit plant photosynthesis (Bradford and Hsiao, 1982). It has shown that photosynthesis reduction in such conditions is associated with malfunction of biochemical reactions (Graan and Boyer, 1990; Lauer and Boyer, 1992). The photosystem II (PSII) is highly sensitive to environmental limiting factors and PSII reaction center and its chemical reactions being adversely affected by drought stress (Vazan, 2002; Cornic, 1994; Comic and Briantais, 1991; Liang *et al.*, 1997). There are many constraints during grain filling stage that generally influence cereals yield. For example, photoinhibition may occur during this stage due to an interaction between drought stress, high temperature and radiation levels (Bolhar-Nordenkamp *et al.*, 1991; Powles, 1984). Using the single factor analysis, one would not be able to distinguish between effects of each variable separately

(Osmond *et al.*, 1987). It has been shown that under high radiation conditions, drought stress enhances inhibition of electron transport (Giardi *et al.*, 1996; Lu *et al.*, 2002; Masojidek *et al.*, 1991). Vazan (2002) reported that drought stress reduces variable fluorescence (FV), initiative fluorescence (F0) and quantum yield (FV, FM). Drought stress also caused non-photochemical quenching to be increased and photochemical quenching to be decreased (Lu *et al.*, 2002). Chlorophyll fluoresces measurement is a non-destructive non-time consuming and relatively simple technique for studying the equilibrium between metabolic and energy evolving processes, that maybe affected by both temperature and drought stresses (Ali-Dib *et al.*, 1994; Araus and Hogan, 1994; Flagella *et al.*, 1994). FV/FM in a previously dark-adapted leaf that is subjected to light, shows potential or maximum quantum yield in PSII. This value varies between 0.75 to 0.85 in non-stressed plants (Baker and Hellon, 1987; Bolhar-Nordenkamp *et al.*, 1989) and show a close correlation with net photosynthesis

quantum yield in intact plant leaves (Comic and Briantais, 1991; Demmig and Bjorkman, 1987; Vazan, 2002). The extreme photosynthetically photon flux density (PPFD) is an important factor affecting quenching in FV/FM ratio under normal conditions (Lu *et al.*, 2002). Declining slope of FV/FM is a valuable criterion for evaluation of photoinhibition in plants that are subjected to environmental stresses, such as drought and high temperatures accompanied with high radiation intensity (Angelopoulos *et al.*, 1996; Havaux *et al.*, 1987; Schreiber and Bilger, 1993). It is important to know FV/FM quenching had risen from an increase in F0 or variations of other components. For example, when all reaction centers are open and photochemical quenching is at minimum, any increase in F0 indicates increase of fluorescence and destruction or malfunction of PSII reaction center, or disruption in electron transport for excitation of reaction centers, but FM quenching may result from increase of non-photochemical quenching (Bolhar-Nordenkamp *et al.*, 1989). In general, it can be concluded that photoinhibition is responsible for all of this changes (Boyer *et al.*, 1987). Although chlorophyll fluorescence was considered as a useful tool for screening and breeding of wheat cultivars under dry conditions (Flagella *et al.*, 1995; Havaux and Lannoye, 1985; Henley *et al.*, 1991; Powles, 1984) and also for high temperature resistance (Osmond *et al.*, 1987), but it may not be useful for other breeding projects. It is believed it should be used to gather with other methods to address drought tolerance (Flagella *et al.*, 1995; Yang *et al.*, 1996). According to Moffatt *et al.* (1990) simulating field conditions in a controlled environment is very difficult, whereas screening in field is a task work. Chlorophyll fluorescence measurement in field indicates actual response of photosynthetic system that is more restricted under natural condition (Araus and Hogan, 1994; Bilger *et al.*, 1995) However, other scientists reported that plants normally face with maximum stress during grain filling, in which environmental factors can adversely affect flag leaf photosynthesis, that supply most of grain required photosynthate (Flagella *et al.*, 1994). The prime objective of this study was evaluation of chlorophyll fluorescence in various wheat cultivars under field-induced drought conditions and studying relation between grain yield with FV/FM, F0, FV and ($T_{1/2}$) in wheat flag leaves experiencing different irrigation regimes.

MATERIALS AND METHODS

The study was carried out at the Experimental Station of Islamic Azad University of Karaj (35°45' latitude and 51°06' altitude) during 2003-2004 growth season. Treatments were different irrigation regimes and wheat cultivars, arranged as a factorial experiment

based on a Randomized Completely Block Design (RCBD) with 4 replications. Soil had a sandy loom texture with EC = 2.3 ds. m⁻² and pH = 7.3.

Irrigation scheduled as irrigation at (T_1) 40% moisture depletion, through growing season (control), (T_2) 60% moisture depletion, from tillering to end of season, (T_3) 80% moisture depletion, from tillering to end of season, (T_4) 60% moisture depletion at flowering and continuing with adequate irrigation, (T_5) 60% moisture depletion, from flowering to the end of season, (T_6) 80% depletion at flowering continuing with adequate irrigation and (T_7) 80% depletion from flowering to the end. Wheat cultivars including two Iranian (Chamran (V_1), Marvdasht (V_2)) and one french (Gaspard (V_3)) were sown with a plant density equal to 400 plant m⁻² with 15 cm row spacing). Fertilizing was done based on results of soil analysis (a total of 100 kg P₂O₅ ha⁻¹ and 60 kg N ha⁻¹ at sowing date in addition to another 60 kg N ha⁻¹ at tillering). In order to determine water movements between plots, some plots with a size similar to experimental plots were constructed before the main experiment with different distance between them (from 50 to 200 cm) and then were irrigated. The moisture content inside and between plots was monitored using chalk blocks and according to obtained results, it was considered a distance equal to 1 m between plots and 2 m between replications. For each replication, also a separate drainage was considered. All plots were irrigated using an installed pipeline system and the volume of water input to each plot was controlled using an automatic and adjustable counter (numerators) and soil moisture content was monitored by wet HH2 continuously. First irrigation was scheduled on 1 October just after planting. Based on results of HH2 and due to frequent precipitations, no more irrigation was required until tillering stage. After that, irrigation was done according to defined treatments and plots were covered with a plastic sheet during rainfall.

The fluorescence parameters were measured in field using a portable plant stress meter and then F0, Fm, Fv, Fv/Fm and $T_{1/2}$ were determined. All measurements were done 20 days after flowering, from 10 am to 2 pm, on flag leaves of three plants in each plot. Leaves were treated by a Photon Flux Density (PFD) equal to 400 $\mu\text{mol photon m}^{-2}\text{s}^{-1}$ for 5 sec and then 20 min of darkness. All measurements were conducted in upper part of flag leaves.

After taking fluorescence records on the leaves, they were transferred to laboratory to determine chlorophyll and relative water content. Chlorophyll content was determined by Froze and Archioze method (Ferus and Arkosiova, 2001). This method implies measurement of absorbed light in plant extract at 647 and 663 nm. Then, chlorophyll content can be calculated by following formula:

$$\text{Chl.a (mg L}^{-1}\text{)} = (12.25 * A_{663}) - (2.79 * A_{647}) * D \quad (1)$$

$$\text{Chl.b (mg L}^{-1}\text{)} = (21.5 * A_{647}) - (5.1 * A_{663}) * D \quad (2)$$

$$\text{Chl.a+b (mg L}^{-1}\text{)} = (7.15 * A_{663}) + (18.71 * A_{647}) * D \quad (3)$$

Where, Chl.a, Chl.b and Chl.a+b represent chlorophyll a, b and their total content, respectively, expressed as mg L⁻¹. A indicates absorbed irradiance by extract at related wavelengths and D is outer thickness of spectrophotometer cuvette in cm finally, the flag leaf chlorophyll content is determined as:

$$\text{PC (mg m}^{-2}\text{)} = (V/1000 * 1/A) * \text{Chl (mg L}^{-1}\text{)} \quad (4)$$

In which, PC is flag leaf chlorophyll content (mg m⁻²), V is consumed volume of acetone 80% and Chl is chlorophyll content calculated from earlier formula.

At maturity, plants were harvested and their yield and yield components were determined for each treatment, separately. Statistical analysis was done using SAS software, in GLM, ANOVA and REG models. Means were compared using Duncan's multiple range test.

RESULTS AND DISCUSSION

Irrigation regimes: Results indicated that irrigation treatments influenced Chl, RWC, CO₂, FV, FV/FM and T_{1/2} significantly, with any significant effect on F0 and FM in flag leaf (Table 1). There was not also any interaction between drought stress and cultivar on determined parameters, excluding chlorophyll content (Table 1). The highest grain yield equal to 8.4 t ha⁻¹ was recorded in control (T₁), with increasing drought stress caused a progressive and significant yield reduction. Therefore, irrigation at 80% soil moisture depletion, from Tillering to the end of growth (T₃) produced the lowest grain yield (53% reduction compared to control), which had no significant difference with irrigation at 80% depletion from flowering to the end of growth (T₇, Table 2).

The chlorophyll content of flag leaves in moderate water-limitation treatments (T₂, T₄ and T₆) did not differ significantly from control (T₁) and in average was up to 25% more than severe treatments (T₃ and T₇, Table 2). These findings are in agreement with Araus *et al.* (1998) that who reported the severe drought treatment in average between three different wheat cultivars caused a 20% reduction in flag leaves chlorophyll content.

The difference of available water between different irrigation regimes during grain filling was evaluated by measuring Relative Water Content (RWC) of flag leaf at 2-3 weeks after flowering. Flag leaf RWC in control plants (T₁) was about 13% higher than severe stressed plants (T₃). This difference in RWC between treatments may

explain existent variance of grain yield and flag leaf chlorophyll content over various irrigation regimes (Table 2). According to Di Marco *et al.* (1988) Stomata closure in the first consequence of drought stress, which results in yield reduction due to disruption of photosynthesis. Irrigation treatments also influenced fast chlorophyll fluorescence parameters in flag leaves (Table 2). Results showed that third irrigation schedule (T₃) induced minimum FV, FM, FM/FV and T_{1/2}, while F0 was similar between irrigation regimes. These findings are in agreement with other reports (Araus *et al.*, 1998; Flagella *et al.*, 1994; Lu *et al.*, 2002); however, Araus *et al.* (1998) observed the highest F0 values in stressful condition, which indicates destruction of PSII reaction centers by drought stress. It is also possible that an interaction between drought, radiation and high temperature stresses in theirs experiments has been caused F0 to be increased under drought condition. In our study, environment temperature was about 25°C and possibly interaction between different stresses had a less effect on PSII reaction centers and plants did not experience heat stress, resulted in a unaffected F0, while 25% reduction of FV was observed in T₃ compared to check plot. Flagella *et al.* (1994) also found no significant difference in F0 between leaves floated in water and those were subjected to drought condition in 25 wheat varieties. Havaux *et al.* (1985) noted drought stress did not induced significant changes in F0 *per se* and heat stress, solely or in combination with drought stress, can induced destruction or disruption of PSII reaction centers and as a result, F0 would be increase.

Whereas F0 values are related to chlorophyll fluorescence of PSI receptors (Anonymous, 1993a; Wilson and Greaves, 1993) and considering non-significant F0 difference between irrigation regimes, it seems the receptor's chlorophylls had almost a similar efficiency between irrigation regimes. As chlorophyll content was decreased with increase in drought severity, it should be partly responsible for photoinhibition. Under drought stresses, recovery of material especially nitrogen will interrupt (Pastore *et al.*, 1989) and furthermore, chloroplasts need to nitrogen to generate chlorophyll through proteins and under nitrogen- or water- limited condition, chlorophyll production rate become slower and as a results, leaves will become more susceptible to photoinhibition (Lauer and Boyer, 1992).

FV value in T₃ showed a 25% reduction compared to control. The fluorescence value of chlorophyll a is generally high when the electron receptor Q is in reduction state; therefore, FV also would be high in this situation. Nevertheless, when the fluorescence value of chlorophyll a is low, Q is in oxidation state and as a result

and FV is decreased. On the other hand, Q is in oxidation state under moisture stress condition; therefore, it may be concluded that drought stress possibly can disrupt normal electron transfer in photolysis at PSII, while has a negligible effect on electron transfer flow (ET8) after first electron receptor (Q). Altogether, water limited condition caused to quantum efficiency of net photosynthesis is declined. Environmental stresses reduce FV value via inhibition of PSII photo-oxidation (Yang *et al.*, 1996). Since FV indicates full reduction of electron receptor (Q), thus it may be accepted that drought stress has disturbed electron transfer to PSI.

FV/FM declining trend from stress conditions was much less compared to fluorescence (Table 2). Since F0 was constant over all irrigation levels, therefore FV/FM declining is related to FM. FV/Fm value is an indicative of PSII capacity to transfer electron (Demmig and Bjorkman, 1987), which has a high correlation to quantum efficiency of net photosynthesis (Anonymous, 1993b). It may be concluded that such reduction in FV/FM ratio may represents protective mechanism of light absorption (Araus *et al.*, 1998) and that drought stress affect photosynthesis efficiency in a lower extent. Araus *et al.* (1998) also found same results.

There was not any significant difference between control and highly stressed plots in respect to $T_{1/2}$ (Table 2). As $T_{1/2}$ is proportional to electron acceptors capacity being on PSII reduction site (24), therefore limited irrigation stress did not influence capacity of electron receptors (PQ) significantly.

Cultivars: Results revealed significant differences between wheat cultivars in respect to grain yield, chlorophyll content, RWC and $T_{1/2}$ (Table 1). Gaspard (V_3) produced the highest grain yield than other two cultivars (6.1 t ha^{-1}) and Chamran (V_1) had the lowest chlorophyll content (Table 2). Gaspard also had the highest RWC.

$T_{1/2}$ in Gaspard was 122.3 ms, significantly higher than Marvdasht and Chamran (Table 2), which indicate the higher capacity of electron receptors in Gaspard compared to other cultivars. As a result, it is expected

more NADPH and ATP production and higher yield in Gaspard. On the other hand, as mentioned before, RWC in this cultivar was same to Marvdasht and higher than Chamran; therefore stomata departure was higher due to higher leaf cell turgescence and more CO_2 entrance. These events can explain higher photosynthate production and grain yield in this cultivar. These findings are confirmed by Araus *et al.* (1998) who reported same relations in their studies with different wheat genotypes. They found a strong correlation between RWC, stomata conduction and CO_2 absorption. The genotypes with higher the RWC, also had lower stomatal resistance and higher CO_2 assimilation and grain yield.

F0 was not significant different between wheat cultivars, that show non significant differences in antenna chlorophyll efficiency (Anonymous, 1993b; Yang *et al.*, 1996) between cultivars. Insignificant variations of FM, FV, and FV/FM ratio over different cultivars (Table 1) reveals similarity of quantum yield in them. It may be therefore concluded that these cultivars had similar quantum yield and observed yield differences can be attributed to capacity of electron acceptors ($T_{1/2}$), which varies in relation to chlorophyll content. On the other hand, Gaspard had a longer growth period; therefore, its higher yield was due to taking advantage from extended growth period, not to its quantum yield.

Correlation between grain yield and chlorophyll fluorescence: Except in case for F0, there was a high correlation between grain yield and other fluorescence parameters (Table 2 and 3). Thus, it seems FV, FM and Fv/Fm are more reliable characteristics to use for evaluation of genotypic differences in drought stress tolerance. However, in study of Araus *et al.* (1998), F0 with Fv and Fm, had the strongest correlation with grain yield, with a weak correlation for Fv/Fm. Contrary to our findings and those of Araus *et al.* (1998) in field condition, Moffatt *et al.* (1990) in their growth chamber experiment with wheat plants found a negative correlation between grain yield and Fv. In general, it seems it is possible using fluorescence parameters to evaluate grain

Table 1: Analysis of variance for chlorophyll fluorescence parameters, leaf chlorophyll content (CHL), relative water content (RWC) of flag leaf and grain yield (GY)

SOV	df	Mean square of measured parameters							
		F0	FM	FV	FV/FM	$T_{1/2}$	CHL	RWC	GY
REP	3	0.10128 ^{ns}	0.421 ^{ns}	0.014 ^{ns}	0.002 ^{ns}	2.877 ^{ns}	1362.5 ^{ns}	137.1 ^{**}	9.44 [*]
T	6	0.0238 ^{ns}	1.438 ^{ns}	0.068 [*]	0.148 [*]	6.615 [*]	8151.3 ^{**}	124.2 ^{**}	79.39 ^{**}
V	2	0.0712 ^{ns}	0.379 ^{ns}	0.013 ^{ns}	0.010 ^{ns}	28.189 ^{**}	4509.9 ^{**}	288.0 ^{**}	16.430 ^{**}
T*V	12	0.0493 ^{ns}	0.470 ^{ns}	0.018 ^{ns}	0.027 ^{ns}	3.16 ^{ns}	2125.6 ^{**}	5.8 ^{ns}	1.667 ^{ns}
ERROR	60	0.0403	0.822	0.026	0.054	2.524	578.8	29.1	2.467
CV%	-	3.6	12	21.8	4.5	3.8	7.16	7.46	9.3

ns, *and **means non-significant, significant at 5 and 1% levels of probability, respectively

Table 2: Mean comparisons of main effects for chlorophyll fluorescence parameters, leaf chlorophyll content (CHL), Relative Water Content (RWC) of flag leaf and grain yield (GY)

		Measured parameters							
Treatments		F0 (ms)	FM (ms)	FV (ms)	FV/FM -	T _{1/2} (ms)	CHL (mg m ⁻²)	RWC (%)	GY (t h ⁻¹)
Irrigation	T ₁	0.32a	1.289a	0.968a	0.746a	112.1ab	364.6a	74.9a	8.4a
	T ₂	0.325a	1.235a	0.910a	0.733a	113.6a	369.5a	71.6ab	4.7de
	T ₃	0.325a	1.048b	0.723b	0.684b	95.8b	295.6c	66.3b	3.9e
	T ₄	0.328a	1.232a	0.904a	0.730a	121.8a	354.5ab	73.3a	6.4b
	T ₅	0.318a	1.196ab	0.878ab	0.727a	106.1ab	316.7bc	76.3a	5.8bc
	T ₆	0.329a	1.192ab	0.863ab	0.721a	113.1ab	346.1ab	72.9a	5.2cd
	T ₇	0.315a	1.167ab	0.851ab	0.720a	109.3ab	303.8c	71.2ab	4.6de
Cultivars	V ₁	0.327a	1.18a	0.896a	0.720a	99.5b	318b	68.9b	5.3b
	V ₂	0.317a	1.18a	0.861a	0.724a	108.9b	344a	73.0a	5.4b
	V ₃	0.325a	1.22a	0.896a	0.728a	122.3a	343a	75.2a	6.1a

Means followed by similar letter(s) in each column are not significantly different

Table 3: Correlation coefficients between grain yield (GY), chlorophyll fluorescence parameters, relative water content (RWC) and chlorophyll content

	F0	FM	FV	FV/FM	T _{1/2}	RWC	CHL	GY
F0	1							
FM	0.39**	1						
FV	0.28 ^{ns}	0.99**	1					
FV/FM	-0.05 ^{ns}	0.88**	0.92**	1				
T _{1/2}	-0.08 ^{ns}	0.35*	0.37*	0.44**	1			
RWC	-0.05 ^{ns}	0.55**	0.58**	0.57**	0.55**	1		
CHL	0.06 ^{ns}	0.46**	0.47**	0.45**	0.55**	0.45*	1	
GY	-0.04 ^{ns}	0.57**	0.60**	0.60**	0.46*	0.58**	0.51**	1

ns, *, **: Non significant, significant at the 5 and 1% levels of probability, respectively

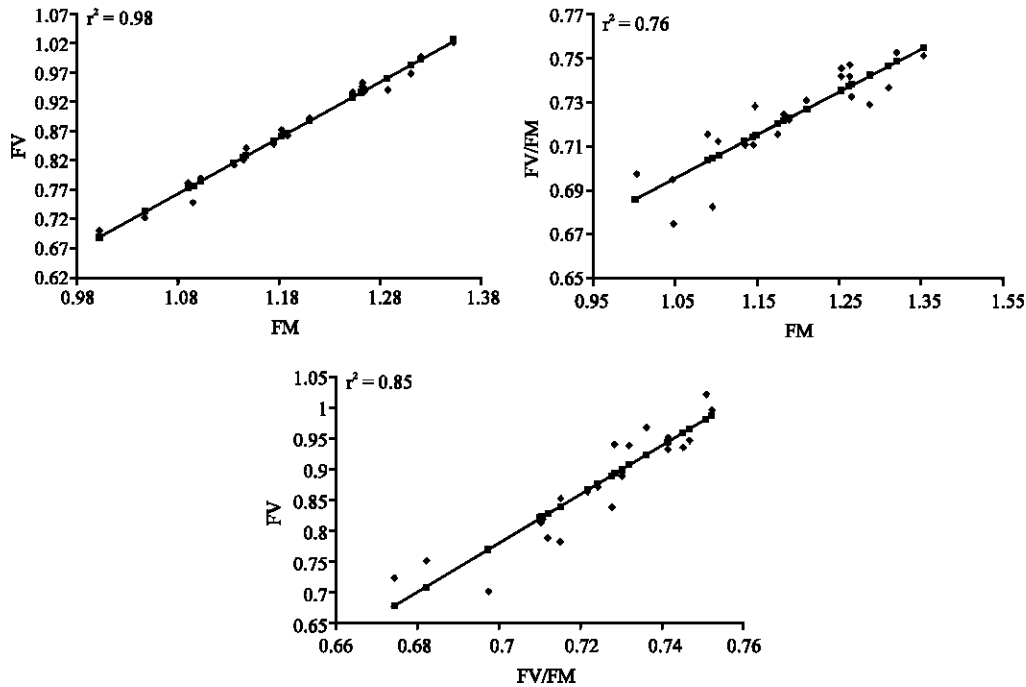


Fig 1: Relation between fluorescence parameters under drought stress

yield of different wheat genotypes under environmental stresses in field, because present results showed that variation in fluorescence parameters is regular (Fig. 1).

Considering above-mentioned findings, occurrence of any significant variation in F0 indicates that strength

of drought stress in this study had not been so high to disturb PSII reaction centers, but Fm reduction under water-limited condition is indicative of less QA oxidation under these situations, that is showing reduction of electron transport between PSII and PSI (Demmig Adams *et al.*, 1989).

CONCLUSIONS

Present results showed that fast fluorescence parameters, measured through grain filling period under deficit irrigation, could be used as a valuable measure to determine stress severity. To do this, Fv, Fv/Fm ratio and even Fm, appeared more suitable criteria than F0. Considering higher $T_{1/2}$ value in higher yielding cultivars, their yield improvement under drought stress has resulted from a more extended grain filling duration, a higher chlorophyll content, a more sustained turgor, or a combination of them. Also according to a non-significant interaction between irrigation regimes and wheat cultivars, treated cultivars showed a similar response to induced drought stress during experiment.

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